Viral hijacking of cellular ubiquitination pathways as an anti-innate immunity strategy.

Mingzhou Chen, Denis Gerlier

Viral hijacking of cellular ubiquitination pathways as anti-innate immunity strategy

Mingzhou Chen[1*] and Denis Gerlier[1]

[1] CNRS ; Université de Lyon ; UMR5537, Laboratoire de Virologie et Pathogenèse Virale ; IFR Laennec ; 69372 Lyon Cedex 08

* Present address: Virology Section, Department of Molecular Biology, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, OH 44195

Running Head: Viral hijacking of ubiquitination

Abbreviations:

APOBEC, apolipoprotein B mRNA-editing enzyme C; APC/C, anaphase promoting complex/cyclosome complex; DDB1, UV-damaged DNA binding; DNA-PK, DNA protein kinase; DUBs, deubiquitinating; E6-AP, E6-associated protein; Hdlg, human homology of the Drosophia melanogaster discs large; HECT, homology to the E6-associated protein carboxyl terminus; HPV, human papilloma virus; hScrib, human homology of the Drosophila scribble; hTERT, the catalytic and rate-limiting subunit of telomerase; ICP0, infected cell protein 0; IFN, interferon; ISG, interferon stimulated gene; KSHV, Kaposi sarcoma associated herpesvirus; MIR1/MIR2, modulator of immune recognition; PHD, plant homeodomain; PML, promyelocytic leukaemia antigen; pRB, retinoblastoma protein; RING, really interesting new gene; SCF, Skp1/Cullin1/F-box; Ub, Ubiquitin; Ubc, ubiquitin conjugating; USP7, ubiquitin-specific protease enzyme; VIF, viral infectivity factor.

Abstract:

Viruses are obligate parasites of host cells. The virus/host coevolution has selected virus for growth despite antiviral defences set up by hosting cells and organisms. Ubiquitin conjugation onto proteins, through a cascade of reaction mediated by the E1 ubiquitin activating enzyme, E2 and E3 ubiquitin conjugating ligases, is one of the major regulatory system which, in particular, tightly control the concentration of cellular proteins by sorting them for degradation. The combined diversity of E2 and E3 ligases ensures the selective/specific ubiquitination of a large number of protein substrates within the cell interior. Therefore it is not surprising that several viruses are coding proteins with E3 ubiquitin ligase activities to target cellular proteins which play a key role in the innate antiviral mechanisms.

Correspondance: Denis Gerlier, CNRS-Université Lyon 1 UMR5537, IFR Laennec, 69372 Lyon Cedex 08, France. E.mail : Denis.Gerlier@univ-lyon1.fr; Tel : +33 4 78 77 86 18 ; Fax : +33 4 78 77 87 54.

Introduction

Viruses have evolved to sneak through the innate and adaptive antiviral response both at the cellular and whole organism levels, for survival and successful infection spreading (29, 38, 57). Most aspects of the life cycle of viruses critically rely on the specific interaction between viral and host cell proteins to redirect the cellular metabolism for their benefit. Post-translationally polypeptide tagging by the conjugation of ubiquitin (ubiquitination), sumo (sumoylation), Nedd8 (neddylation) and ISG15 (ISGylation) (52, 124) is a potent way to alter protein function and/or to sort protein. The ubiquitin-proteasome system is a mandatory player in many regulatory processes in mammalian cells (39). Monoubiquitination of proteins are sorted and polyubiqitinated proteins are targeted for degradation into small peptides by the 26S proteasome. The latter is necessary to ensure efficient turn-over of most cellular proteins. Elegantly, the evolution has selected for the screening of short peptides derived from proteasome degradation as a read-out of self integrity via the MHC class I presentation pathway to CD8 T lymphocytes.

Ubiquitin (Ub) is a conserved 76 amino acid polypeptide when attached to a protein mediates interaction with other proteins (53). Ubiquitin conjugation to a substrate involves a cascade of at least three different enzymatic reactions. In the first step, the ubiquitin binds to the C-terminus of E1 activating enzyme by a thioester linkage through an energy-requiring process. In the second step, activated Ub is transferred, again through a thioester linkage, to an ubiquitin conjugating enzyme E2 (Ubc) or E2 ligase. In the third step, the activated ubiquitin is transferred from the E2 thioester linkage to a lysine residue of the target protein, through a peptide-bond onto the side chain, resulting in a branched peptide. This last step is catalyzed by an Ubc E3 or E3 ligase, which specifically recognizes the substrate proteins. Ubiquitination can reversibly take place through the action of deubiquitinating (DUBs) enzymes, which remove ubiquitin chains from specific ubiquitin-protein conjugates (5, 23, 53, 124). Thus ubiquitination follows dynamic forward and backward processes. In human, the Ub enzymatic players are unique for E1, over 50 for E2 and several hundreds for E3. The large number of E2 and E3 ligases and their combination ensures the necessary specific and individual targeting of thousands of different proteins.

Structurally and functionally, E3 ligases are heterogeneous. One group, which includes the Nedd4 family, is characterized by the presence of the homology to the E6-associated protein carboxyl terminus (HECT) catalytic domain, (58). These are the only catalytic E3 ligases on which activated Ub is transferred from E2, again through a thioester bond, before Ubiquitin transfer to the target substrate through a peptide bond. The prototype is the E6-associated protein (E6-AP). The second group acts only as a linker or a scaffold to bring specific substrates near Ub charged E2 ligase closer enough to enable the Ub transfer from E2 to a Lys residue of the substrate. This group can be subdivided into unimolecular and multimolecular E3 ligases. Unimolecular linker-type E3 ligases contain a Zn-finger called the really interesting new gene (RING) domain which recruits E2 enzymes. RING domains are closely related to PHD domains (or PHD fingers) and the frontier is disputed among the structuralists, the issue of which is the prediction of E3 ligase activity (109). The U-box is found as an alternative to the RING domain. U-box is predicted to be structurally related, but lack the hallmark metal-chelating residues (50). The prototype of unimolecular RING E3 ligase is MDM2, the major E3 ligase of p53. The multicomponent E3 ligases contain a variable number of subunits with at least one subunit characterised by the presence of a RING domain and a complex containing one Cullin protein. The RING domain is responsible for the recruitment of E2 and the Cullin complex acts as a scaffold for the recognition of specific substrates. Prototypes of multisubunit E3 ligases are the SCF (4) and anaphase promoting complex/cyclosome (APC/C) complex (17).

Because of the necessary continuous adaptation of viruses to their hosts, it is not surprising that viruses can modify the ubiquitin-proteasome machinery of host cells and use it for their own profit. So far, this modulation process takes place at the E3 ligase level i.e. at the step where the substrate specificity is critically defined. Some viral proteins acts as E3 ligases, and other redirect host ubiquitin E3 ligases to target new substrate proteins (5). Viral E3 ligases are involved in the regulation of many aspects of viral and cellular processes such as virus budding, cell division, apoptosis, antigen presentation, lymphocyte activation, induction of T cell-tolerance, immune evasion, and innate immunity to list a few (5, 75).

The scope of this review is to focus on viral hijacking of Ubiquitin ligases to modulate cellular intrinsic antiviral activities and innate immunity. Based on a classification of E3 ligase according to their catalytic/non catalytic activity, and on their unimolecular or multisubunit structure, the following viral ubiquitin E3 ligases will be reviewed: RTA, a novel unimolecular catalytic E3 ligase, E6, a E3 ligase able to hijack another (catalytic) E3 ligase, ICP0 a bifunctionnal unimolecular RING-type E3 ligase, E4orf6/E1B55K and VIF, two RING/Cullin E3 ligase "BC-box" subunits, and V, a RING/Cullin E3 ligase subunit with a new Zn-finger motif.

1) Kaposi sarcoma associated herpesvirus RTA protein: an unimolecular viral catalytic E3 ligase

Kaposi sarcoma associated herpesvirus, KSHV, is a DNA tumour virus that cause rare endothelial and lymphoid tumour mostly in immunocompromised patients. The viral RTA protein is a DNA binding nuclear transcription factor acting throughout the virus replication cycle.

Gene and structure

KSHV Orf50 codes an protein of 691 amino acid length, called RTA, which is a homolog of the RTA protein coded by Epstein Barr virus, another oncogenic *herpesviridae* (116). It was found to bind to IRF7 during a yeast two hybrid screening of a human cDNA library.

E3 ligase activities

RTA amino-terminal half-part binds to IRF7 (FIGURE 1) and induces its polyubiquitination and degradation by the proteasome. In vitro, RTA acts as a unimolecular E3 ligase for ubiquitination of IRF7 in the presence of the Ubch5α E2 ligase, E1 and ubiquitin. RTA also recognises itself as a substrate for polyubiquitination. RTA has a Cys-rich region of a novel type which is proposed to harbour the intrinsic catalytic E3 ligase activity. Indeed mutations of key Cys or His residues within this region result in the loss of E3 ligase activity of RTA without hampering its binding to IRF7 (127).

Cellular impact and counteraction of innate immunity

Besides the key role of RTA in the positive regulation of viral transcription (see (127) and references therein), RTA is predicted to counteract the innate immunity by preventing the activation of IFN-α gene. Indeed IRF7 is a key transactivator of this gene (95). Interestingly, KSHV code for at least two other proteins with E3 ligase activity, MIR1 and MIR2. They are involved in the regulation of the adaptative immunity, because they target MHC class I molecules for degradation (24).

2) Human papillomavirus E6 protein: hijacker of a unimolecular E3 ligase

The high risk human papillomaviruses (e.g. HPV-16 and HPV-18) are causative agents of cervical cancers. Their oncogenic properties correlate with the transforming activities of the viral oncogenes E6 and E7. Both of them use the ubiquitin-proteasome system to target a variety of important negative cell regulatory proteins. E7 protein upregulates proliferation-related genes by interacting with the retinoblastoma protein pRb, and related protein p107 et p130 (31), (see also (5, 110) for review). E6 circumvents the cell apoptotic response to uncontrolled cell proliferation by binding to p53 (123), see also (67) for review.

Genes and structures

E6 and E7 are two early transcribed genes located first after the unique viral transcription promoter. E6 and E7 are relatively small proteins with a size of about one hundred and one hundred and fifty amino acids, respectively. Non oncogenic HPVs differ from the oncogenic HPV-16 and HPV18 by encoding E6 and E7 proteins poorly efficient in recruiting their cellular targets for degradation by the ubiquitin and proteasome pathway (26, 41, 107).

E3 ligase activities

E6 protein displays two types of E3 ligase activities according to the involvement or not of the cellular E6-AP protein (FIGURE 2).

E6-AP dependent E3 ligase

On one hand, E6 binds through its N-terminus to the unimolecular E6-AP E3 ligase. E6-AP contains an active enzymatic HECT site which interacts with several E2 conjugating enzymes, including UbcH5, UbcH6, UbcH7 and UbcH8 (see (110) for review). On the other hand, E6 recruits many cellular proteins as substrates for ubiquitination.

E6 oncoprotein promotes the degradation of p53 through its interaction with E6-AP to form an E3 ubiquitin ligase complex (55, 117) (see also (110) for review). Firstly, E6 associates with E6-AP, secondly, the dimeric E6/E6-AP complex binds to p53 and induces E6-AP-mediated ubiquitination of p53, and thirdly, polyubiquitinated p53 is recognized and degraded by 26S proteasome (see (110) for review). E6 association with E6-AP likely alters its substrate specificity because E6-AP itself is unable to recognize p53 as a target for ubiquitination (117). Conversely, does E6 binding to E6-AP prevent its activity on normally E6-independent substrates ? The precise scaffold of the E6/E6-AP/p53 complex is yet to be uncovered. It is proposed that a small helical domain within E6-AP (L2G motif), which binds to E6, also associates with p53 (56), and E6 binds to the core DNA-binding domain of p53 (44, 96). Strikingly, the effect of E6 on p53 is independent of the six C-terminal lysine residues in p53, which are critical for effective ubiquitination mediated by the physiological cellular unimolecular RING-type E3 ligase Mdm2 (15).

HPV E6 proteins also promote the E6-AP-dependent degradation of many other proteins (see (37, 110) for review) that are independent of p53 degradation, including E6-AP (59), the human homolog of the Drosophila melanogaster tumor suppressor Discs large (hDLG), the human homolog of the Drosophila Scribble (Vartul), the apoptosis-promoting Bak protein, a novel GAP protein called E6TP1, MAG-1, the DNA repair protein, O(6)-methylguanine-DNA methyltransferase MGMT, MUPP-1, the GAIP(GTPase-activating protein for Gαl)- interacting protein C terminus TIP2/GIPC (36) and two PDZ containing proteins, hScrib, a tumor suppressor protein, and NFX1-91, a cellular repressor of human *hTERT* (the catalytic and rate-limiting subunit of telomerase) (72). E6 also binds to, and can ubiquitinate c-Myc (42), although this latter event is not observed in physiological conditions (122).

E6-AP independent E3 ligase

E6 is also an E3 ligase in the absence of E6-AP for several substrates including Blk, a member of the Src-family of non-receptor tyrosine kinase, Bak, a human proapoptotic protein, Mcm7 and two human homologues of the yeast DNA repair protein RAD23, HHR23A and HHR23B. The mechanism by which E6 targets proteins for degradation in an E6-AP-independent manner is presently unclear (see (5, 110) for review).

Cellular partners but not substrates of E3 ligase

E6 interacts with another set of cellular proteins without evidence for ubiquitination and degradation including E6-BP (21), CBP/p300 (94, 130), Tyk2 (65), the transcriptional integrator of the E2F1/DP1/RB cell-cycle regulatory pathway TRIP-Br1 (45) and IRF-3 (103). The binding site of these partners are unknown, but there is evidence for multiple binding sites on P6 including its PDZ binding domain.

E7 protein: a substrate recruiting sub-unit of an E3 ligase?

The ability of oncogenic HPVs to target cellular proteins for proteasome-mediated degradation is not restricted to E6. E7 is a substrate for the UbcH7 E2 and Cul1-Skp2 containing E3 ligases (89). E7 binds to pRb and related proteins (5, 110) and induces their ubiquitination and degradation by the 26S proteasome. These data suggest that E7 may also act as a substrate recruiting sub-unit of a complex E3 ligase.

Cellular impact and counteraction of innate immunity

Besides their strong impact on the cell cycle control, apoptosis and oncogenic properties which are the subject of intensive work, E6 and E7 proteins exhibit multiple anti-interferon activities (62). Surprisingly the inhibitory effect of E6 and E7 is not related with their ability to target cellular protein for ubiquitination and degradation. E6 binds to CBP, P300 and IRF-3 and inhibits their transcriptional activity (94, 103). Since IRF3 and CBP/p300 are cooperative subunits

of the IFN-β enhanceosome expression of E6 blocks IFN-β gene activation upon viral infection (103). E6 binds to Tyk-2 and competes for Tyk-2 binding to the interferon receptor subunit IFNAR1. Thus, E6 inhibits the downstream activation of the Jak-STAT1-STAT2 pathway (65), and cells poorly respond to exogenous IFN treatment. Further downstream of this pathway, E7 binds to IRF9 and inhibits the transcriptional activity of the ISGF3 enhanceosome made of IRF9, STAT1 and STAT2 (6, 7). Thus, altogether, E6 and E7 block both the activation of the type I IFN gene and the IFN activation of the innate antiviral immunity as shown by the severe down regulation of IFN-responsive genes (85). Last but not least, since apoptosis induced by IFN-α/β depends upon p53 (97), the E6-mediated degradation of p53 further contributes to prevent death of the virus infected cells.

3) Herpes simplex virus ICP0: a dual E3-Ubiquitin ligase

Herpes simplex virus-infected cell protein 0 (ICP0, also called vmw110) was initially described as a protein found to accumulate in infected cells, but not present in the virion. It acts as a promiscuous transactivating signal, since expression by transfection results in the activation of numerous cellular genes (see (46) for review).

Gene and structure

ICP0 is coded by α0 which is transcribed in several spliced mRNA subspecies. The 775 aa long protein is extensively post-translationally processed and the pattern of isoform expression vary with the progress of the infection. It contains a nuclear localisation signal and a self-interacting domain leading to formation of dimers and higher ordered multimers.

E3 Ubiquitine ligase activity

ICP0 dynamically interacts with the proteasome (121) and is the only known ubiquitin ligase protein exhibiting two independent E3 sites (47). ICP0 has a RING domain and a HUL-1 domain close to its NH_2 and COOH terminus respectively (FIGURE 3).

The RING domain is responsible for the recruitment of both of the cellular E2 ubiquitine conjugating enzyme UbcH5a (13) and one cellular substrate, the ubiquitin-specific protease enzyme USP7 (also called HAUSP) (35).

ICP0 is its own substrate for ubiquitination (16). It also directly ubiquitinates USP7 in vitro and in vivo, and, this activity leads to a reduction in cellular USP7 levels during HSV-1 infection (35). Conversely, USP7 stabilizes ICP0 in vitro and in vivo by protecting ICP0 from auto-ubiquitination (16). These reciprocal activities of the two proteins mimic the USP7-mediated stability of Mdm2 (64). The outcome during productive HSV-1 infection is that the USP7-mediated stabilization of ICP0 is dominant over ICP0-induced degradation of USP7 (10).

The ICP0 mediated ubiquitination of p53 is weak compared to that of Mdm2, the major cellular E3 ubiquitin ligase which keeps the p53 in low level in uninfected cells. ICP0 binds to p53 by residues 241 to 594 and then promotes low levels of p53 ubiquitination in infected cells (11).

Other cellular proteins targeted for proteasome-mediated degradation by the ICP0-UbcH5a complex are the catalytic subunit of DNA protein kinase (DNA-PK) (93), the centromeric proteins CENP-C and CENP-A (34, 77) and two major components of the nuclear substructure ND10, the promyelocytic leukemia antigen PML (12, 20) and small ubiquitin-like modifier (SUMO)-modified forms of SP100 (20, 92). In cells expressing ICP0, PML can be easily destroyed, but neither PML nor its SUMO-modified forms has been successfully ubiquitinated directly in vitro by ICP0 (12). Thus an additional factor or some unknown substrate may be required to form an active E3 ligase complex for in vivo degradation of PML and/or sp100. In cells expressing dominant-negative UbcH5a, but not dominant-negative UbcH6 or UbcH7, blocks ICP0 RING-mediated PML and sp100 degradation and can delay ND10 disruption by at least several hours (43).

The second ubiquitin ligase domain HUL-1 within ICP0, is not a Zn-finger and is required for the ubiquitination of the E2 ubiquitin ligase UbcH3 (cdc34) (121). UbcH3 is the major E2

enzyme which forms a complex with skp1-skp2-F-box and promotes the degradation of cyclin D1 and cyclin D3 (see (25) for review). ICP0 was found to stabilize both cyclins D3 and D1, without evidence for a direct interaction with cyclin D1 (121). UbcH3 strongly interacts with ICP0 20-241 region, which encompasses the RING domain, and moderately to ICP0 621-625 or HUL-1 domain (47). Only the latter domain and aspartate 199 are essential for ubiquitination and degradation of UbcH3, since, in cells infected with HSV-1, ICP0 with disrupted RING domain has no effect on UbcH3 degradation. Thus, N-terminus of ICP0 would indirectly contribute to the ubiquitination of UbcH3 by capturing it and pushing it towards the second ligase activity site (46).

Cellular impact and counteraction of innate immunity

Owing its numerous substrates and multiple molecular partnerships, ICP0 interferes with many viral and cellular functions. ICP0 with intact RING finger stimulates lytic infection and reactivates quiescent HSV-1 viral genomes (see (46) for review). HSV-1 mutants devoid of ICP0 are less cytotoxic and less pathogenic. Disruption of kinetophore due to polyubiquitination of CENP subunits by ICP0 results in abnormal chromosome segregation, unusual cytokinesis, and nuclear morphological aberrations: cells become stalled at an unusual stage of mitosis defined as pseudoprometaphase (34, 54, 76). However, the impact of ICP0-mediated UbcH3 degradation and resulting cyclin D1 and D3 stabilization remains unclear in HSV infected cells (33).

ICP0 is clearly involved in the dampening of the (i) development of antiviral state and (ii) the amplification through the IFN⇔IFNAR pathway.

(i) During infection by HSV-1, there is little expression of interferon stimulated genes (ISGs), whereas cells infected by mutant ICP0^{null} HSV-1 exhibit high level of ISG expression (32). A significant part of this ISG expression is likely independent from the elicited IFN response since it is insensitive to a protein synthesis inhibitor (82, 87, 99). ICP0 acts by inhibiting IRF-3-mediated activation of ISGs (69, 81). This inhibition critically relies on intact RING domain and active proteasome-dependent proteolysis (32, 69). IRF-3 turn-over is increased and nuclear accumulation of IRF-3 is blocked by ICP0 (81), but ICP0 does not induce the degradation of TBK1, IRF-3, IRF-7, or CBP which all belong to the IRF-3 signalling pathway (69).

(ii) While wild type HSC-1 is relatively insensitive to exogenous interferon α/β treatment of host cells, the growth of ICP0^{null} HSV-1 is inhibited in Vero cells pretreated by type I interferon (49, 83, 84). Moreover, mutant ICP0^{null} HSV-1 poorly replicates in mice, a phenotype which is reverted in IFNAR$^{-/-}$ mice (63).

How does E3 ubiquitin ligase activity of ICP0 can contribute or even be responsible for the ICP0 blocks of the induction of an antiviral state ?

(1) The ICP0 mediates the degradation of PML which is required for the interferon response. Indeed, exogenous IFN does not induce an efficient antiviral state in PML$^{-/-}$ cells and does not affect the growth of ICP0^{null} HSV-1 in these cells (19). Interestingly, CBP/p300, which are subunits of the enhanceosome downstream to the IRF-3 pathway, bind to PML (106) and their nuclear distribution is strongly modified in HSV-1 infected cells provided that ICP0 with an intact RING domains is expressed (69).

(2) DNA-PK stabilizes IRF-3 (60), and ICP0-mediated targeting of DNA-PK for degradation may contribute to the weakening the IRF-3 activation pathway.

(3) P53 is up-regulated by IFN to mediate apoptotic signal (97). ICP0 mediated targeting for degradation of p53 can contribute to the resistance of HSV to IFN.

In conclusion, ICP0 is an E3 Ubiquitin ligase which targets several cellular proteins, some of them being involved in the cellular innate immunity. We propose that the potent anti-innate immunity properties of ICP0 results from the coordinate disruption of several innate immunity pathways. Furthermore, at a late stage of HSV-1 infection, ICP0 prevents the degradation of rRNA according to a new antiviral mechanism distinct from the IFN-induced RNAse L pathway. This effect, however, does not requires an intact RING domain (111).

4) Adenovirus E4orf6 and E1B55K protein: substrate recruiting sub-units of an E3 ligase

Human adenovirus has evolved strategies to regulate cellular proteins function to permit efficient viral replication. The viral E1B-55K/E4orf6 ubiquitin ligase is also required for efficient

322 viral late protein synthesis in many cell types, but the mechanism is not understood.
323
324 ***Genes and structures***
325 E4orf6 and E1B55K are two genes expressed early after adenovirus infection. They
326 encoded a 34 kDa and 55 kDa proteins, respectively. E4orf6 belongs to the virus genes involved
327 in the virus transcription and cell cycle control and E1B55K participates in inhibiting apoptosis.
328
329 ***E3 ligase activities***
330 In productively infected cells, adenovirus E4orf6 and E1B55K redirect the cellular E3
331 ligase complex made of RING protein Rbx1/Roc1, Cullin 5, Elongin B and C (FIGURE 4) to target
332 p53 for polyubiquitination and degradation (1, 18, 48, 100-102, 112) (see also (8, 105) for review).
333 Infection with mutant viruses that do not express either E1B55K or E4orf6 proteins does not
334 induces p53 degradation (112). E4orf6/E1B55K E3 ligase complex is remarkably similar to the
335 Von Hippel-Lindau tumor suppressor and SCF (skp-Cul1) E3 ubiquitin ligase complex. Rbx1
336 interacts with E4orf6 but not with E1B55K (100), and looks acting as a substrate specificity factor.
337 This complex interacts with the E2-conjugating enzyme UbcH3 to conjugate ubiquitin chains to its
338 substrates. E1B55K is the substrate recognition subunit of this complex. Both E4orf6 and
339 E1B55K contain putative BC-box, but only E4orf6 directly interacts with Elongin C via its BC-Box
340 motif. Furthermore, E1B55K also does not bind stably to isolated E4orf6 and requires E4orf6 to
341 be in complex with Cul5 and Elongins B and C. The formation of the complex is thought to alter
342 the conformation of E4orf6 and stabilize the interaction between E4orf6 and E1B55K (9). E4orf6
343 and E1B 55K bind p53 near its N and C termini, respectively. The ligase complex activity is also
344 critical dependent on NEDD8 which modifies the activity of Cullin5 (90, 100, 102). The E2
345 conjugating enzyme UbcH3 (cdc34) is associated with E4orf6 in vivo, and, in an in vitro
346 ubiquitination test, UbcH5 acts as a functional E2 enzyme (100).
347 E1B55K/E4orf6-Elongins B/C/Cullin5/Rbx1 E3 ligase complex can target one or more
348 subunits of the MRN complex involved in DNA double-strand break repair for proteasome-
349 mediated degradation (114), although there is no direct evidence for MRN single subunits to be
350 polyubiquitinated. E1B55K/E4orf6/elonginBC/Cullin5/Rbx1 also exploits the cellular aggresome
351 response to accelerate the degradation of MRN complexes in adenovirus-infected cells (74).
352 Aggresome formation may contribute to protect the viral genomic DNA from MRN activity by both
353 sequestering MRN in the cytoplasm and dramatically promoting its degradation by the
354 proteasome.
355 During the late phase of infection by adenovirus, E1B55K/E4orf6 complex promotes the
356 nuclear export of viral mRNA and prevents that of cellular mRNAs (see (8) for review). Does the
357 E1B55K/E4orf6 E3 ligase complex also target a mRNP protein involved in most cellular mRNA
358 nuclear export and enhances export and translation of late viral mRNA (8) ?
359 E4orf6 can interact with p53 and inhibit its transactivating activity (18, 28). In the absence
360 of E4orf6, E1B55K dramatically increases the concentration of p53 (79). But p53 transactivating
361 activity is blocked. Possibly, upon interaction with p53, E1B55K bring a repression domain close
362 to the p53 activating domain (8). E1B5K also inhibits the acetylation of p53 by PCAF and thus
363 contributes to p53 inhibition by another mechanism (73).
364
365 ***Cellular impact and counteraction of innate immunity***
366 Adenoviruses have developed several genes to control the antiviral effect of innate
367 immunity (see (14) for review). Lowering the p53 contents of the cell by E4orf6 and E1B 55K
368 proteins likely contributes to protect the infected cells from IFN induced p53-dependent apoptosis
369 (97). Furthermore, by blocking nuclear export of cellular mRNA, they may have a major impact on
370 the expression of IFN and ISG genes.
371
372
373 **5) Lentivirus VIF protein: a substrate recruiting sub-unit of an E3 ligase**
374
375 The viral infectivity factor VIF encoded by HIV-1 and most other lentivirus was initially
376 found in the nineties to be required for replication in “non permissive” cells such as primary T cells
377 and macrophage but dispensable for replication in epithelial cell lines. More than ten years later,

the cellular target APOBEC3G was identified (see (104) for review).

Gene and structure

VIF is coded within the region on an alternative codon frame and has a size of about 23 kDa. Functionally, VIF shared many features with the adenoviral E4orf4 protein.

E3 ligase activities

VIF contains a BC-like-box (or SOCS-Box) (126, 128) to recruit Elongin C/B (FIGURE 5). Binding to Elongin-C is negatively regulated by serine phosphorylation of the BC-box (80). VIF does not have a Cul-Box, but contains a HCCH motif (Hx5Cx17-18Cx3-5H), with potency to coordinate a zinc atom, the integrity of which is required for binding to Cullin 5 (78), In addition it binds to the RING containing Rbx1 E3 ligase subunit (126). Vif connects the APOBEC3G and APOBEC3F (apolipoprotein B mRNA-editing enzyme) as a substrate to the multisubunit E3 ligase for polyubiquitination and degradation (71, 126). The active E2 ligase recruited by the RING domain of Rbx1 has not been defined in vivo, although Ubc12 and Ubc5A can work in vitro. The loss of function of VIF mutants correlates with their inability to bind to APOBEC3G or to give rise to functional E3 ligase (61). VIF is also autoubiquitinylated by the same E3 ligase complex which explains is short half-life in vivo (40, 71, 80). Overexpression of APOBEC3G stabilizes Vif expression as if the two substrates compete with each other (71). Thus, VIF functions like an F-box protein by bringing together the Cul5 complex and the substrate.

Surprisingly, APOBEC3G is also monoubiquitinated by the unimolecular HECT-type E3 ligase Nedd4.1, for its efficient packaging within budding virions (30). Thus, APOBEC3G is the substrate for both monoubiquitination and polyubiquitination by two separate E3 ligases.

Besides targeting APOBEC3G for ubiquitination and degradation, VIF may also directly inhibit its deaminase activity, as suggested in experiments performed in *E. Coli* (108).

Cellular impact and counteraction of innate immunity

APOBEC3G is a cytidine deaminase which deaminates cytidine to uracil, resulting in deleterious overmutagenesis of the HIV-1 genome. Furthermore, APOBECG3G displays another anti-HIV-1 activity which is independent from its cytidine deaminase activity (86). Vif activity is a species-specific factor because it cannot recognize APOBEC3G from other species which differ by a single residue within the binding site (D128K) (71). This intrinsic cellular immunity belongs also to the inducible innate immunity since a type I IFN treatment can upregulate the APOBEC3G expression (118).

6) Rubulavirus V proteins: a substrate recruiting sub-unit of an E3 ligase

Rubulavirus are enveloped RNA viruses whose replication occurs entirely within the cytosol. Their genome code for less than ten proteins, nevertheless because they also have to cope with the cellular innate immunity, at least one of them, V protein, is a potent inhibitor of the interferon system. As adenovirus EE4orf6 and Vif, V protein acts a scaffold linking a multi-subunit cellular E3 ligase to new cellular substrates.

Gene and structure

Members of the *Rubulavirus* genus (simian virus 5 -SV5-, human parainfluenza virus 2 -hPIV2- and mumps virus) which belongs to the *Paramyxoviridae* family and *Monogavirales* order have a negative strand RNA whose genome contains 7 genes coding for 8 proteins. Indeed the second gene codes the P protein, a polymerase cofactor, and, upon editing of P mRNA, to V protein. V is two hundred amino acid long, shares a common N sequence with P and has a minor C-terminus rich in Cys residues, a hallmark of all *Mononegavirales* V protein. This C-terminus is a new Zn-finger with no homology with other known Zn-finger structures (66).

E3 ligase activities

Rubulavirus V proteins were initially characterized for their ability to bind to the highly conserved UV-damaged DNA-binding protein DDB1 protein (68) (FIGURE 6). DDB1 has a

multipropeller structure associating three β-propellers called BPA, BPB and BPC and one C-terminal helical domain (66). SV5 V binds to the BPA-BPC double propeller pocket by inserting its N-terminal helix, while the Zn-finger does not interact with DDB1. The BPC propeller DDB1 docks to the N-terminus of the E3 ligase Cul4A scaffold (66). Cul4A can recruit the Rbx1 RING protein (or another protein ?) which in turns recruits a yet to be defined E2 ligase. Mumps V protein binds also to this later protein (120). V proteins from SV5, mumps and hPIV2 multimerize and bind to STAT2. A single residue (Asn100 in V from SV5) located in a β–sheet determined efficient binding to STAT2 (66, 125). Only hPIV2 V can directly target STAT2 as an ubiquitination substrate, although V from SV5 can do so in vitro (98, 120). Instead, STAT2 is used by V proteins from SV5 and mumps as a scaffold to recruit STAT1 which is polyubiquitinated and degraded by the proteasome (3, 27, 119). Mumps V can also recruit directly STAT3 for ubiquitination and degradation, the later process for which the recruitment of Rbx/Roc1 is required (120)

.

Cellular impact and counteraction of innate immunity

STAT1-STAT2 heterodimers associated with IRF9 constitute the critical transactivating complex downstream the signalling induces by IFN binding to IFNAR. By downregulating STAT1, V protein is predicted to render mumps, SV5 and hPIV2 viruses less sensitive to IFN-mediated antiviral effect. Indeed, the inability of V protein to target mouse STAT1 correlated well with the very poor replication of SV5 in mice, whereas $STAT1^{-/-}$ mice are sensitive to viral infection (see (51) and references herein).

However, in vitro, the phenotype of recombinant SV5 virus with C-truncated V protein is complicated, because V exhibits many other functions. (i) It binds to MDA5 (melanoma differentiation-associated gene 5), a companion molecule of the RIG-I-dependant IFN-β activation pathway (2, 51). (ii) V acts as an anti-apoptotic factor (115). Interestingly, all these functions require an intact Zn-finger. (iii) In a minigenome replication model, V protein exhibits transcription and replication inhibition properties (70).

The functional impact of STAT3 degradation by mumps V protein remains to be clarified since the role of STAT3 is variable according to the cell type (113).

Conclusion

We have illustrated, in this review, the various strategies used by viruses to hijack the ubiquitination pathway and target cellular proteins for degradation (or disrupting their function ?) in order to evade cellular innate antiviral response. Can a virus act also by inhibiting cellular ubiquitination ? The answer is probably yes, as revealed by the ability of measles virus P protein to inhibit ubiquitination and stabilize the RING-type E3 ligase PIRH2 protein (a homolog of MDM2), although the physiological relevance of this observation remains to be uncovered (22). This short survey has brought a glimpse of what we predict will be an increasing area of knowledge, namely the subversion or the use of ubiquitination and related peptide conjugation such as sumoylation and ISGylation by viruses to adapt their cell host for optimal replication and survival in the context of a whole organism and population. Indeed, there are numerous cellular E3 ligases, some of which are upregulated by type I IFN (88), and beside the dozen of cellular proteins so far identified as antiviral weapons, there are likely many other cellular proteins which can exhibit non specific or specific antiviral activities. For example, one of the gene is ISG15 which is an ubiquitin-like protein, that, on one hand, targets the release of HIV-1 (91), and, on another hand, has its conjugation property inhibited by the Influenza B virus NS1 protein (129).

Acknowledgement: The authors thank Florence Herschke for her comments.

FIGURE legends

FIGURE 1. Intrinsic catalytic E3 ligase activity of KSV RTA protein.

FIGURE 2. E6-AP independent (upper) and E6-AP dependent E3 ligase activity of HPV E6 protein, known substrates (dot lined), binding (full lined) partners and possible effect on innate immunity. For symbols see FIGURE. 1.

FIGURE 3. Current view of E3 ligase activity of HSV ICP0 protein: known substrates and possible effect(s) on cellular functions. The molecular support for the recruitment of sp100, PML, CENP-A/C and DNA-PK as the substrates for ICP0 E3 ligase activity is yet unknown. For symbols see also FIGURE. 1.

FIGURE 4. E3 ligase activity of adenovirus E4orf6 and E1B55K proteins. E1B55K is stably bound to E4orf6 only when the latter is in complex with Cullin 5 and Elongins B/C. For symbols see FIGURE. 1.

FIGURE 5. E3 ligase activity of HIV-1 Vif protein resulting in self and APOBEC-3G polyubiquitination. Vif is also monoubiquitinated by HECT-type E3 ligase Nedd4-1 which results in the efficient Vif encapsidation into virions. For symbols see FIGURE. 1.

FIGURE 6. E3 ligase activity of Rubulavirus V protein. Mumps V interacts directly with Roc1/RBX1, and recruit STAT3 as ubiquitination substrate. Mumps and SVF5 interacts with STAT2 solely to recruit STAT1 as the ubiquitination substrate. HIPV2-V protein binds and targets STAT2 for ubiquitination. Roc1/RBX1 looks dispensable for STAT1 or STAT2 ubiquitination by any V protein. For symbols see FIGURE. 1.

References

1. **Ali, S. H., J. S. Kasper, T. Arai, and J. A. DeCaprio.** 2004. Cul7/p185/p193 binding to simian virus 40 large T antigen has a role in cellular transformation. J Virol **78:**2749-57.
2. **Andrejeva, J., K. S. Childs, D. F. Young, T. S. Carlos, N. Stock, S. Goodbourn, and R. E. Randall.** 2004. The V proteins of paramyxoviruses bind the IFN-inducible RNA helicase, mda-5, and inhibit its activation of the IFN-beta promoter. Proc Natl Acad Sci U S A **101:**17264-9.
3. **Andrejeva, J., D. F. Young, S. Goodbourn, and R. E. Randall.** 2002. Degradation of STAT1 and STAT2 by the V proteins of simian virus 5 and human parainfluenza virus type 2, respectively: consequences for virus replication in the presence of alpha/beta and gamma interferons. J Virol **76:**2159-67.
4. **Ang, X. L., and J. Wade Harper.** 2005. SCF-mediated protein degradation and cell cycle control. Oncogene **24:**2860-70.
5. **Banks, L., D. Pim, and M. Thomas.** 2003. Viruses and the 26S proteasome: hacking into destruction. Trends Biochem Sci **28:**452-9.
6. **Barnard, P., and N. A. McMillan.** 1999. The human papillomavirus E7 oncoprotein abrogates signaling mediated by interferon-alpha. Virology **259:**305-13.
7. **Barnard, P., E. Payne, and N. A. McMillan.** 2000. The human papillomavirus E7 protein is able to inhibit the antiviral and anti-growth functions of interferon-alpha. Virology **277:**411-9.
8. **Berk, A. J.** 2005. Recent lessons in gene expression, cell cycle control, and cell biology from adenovirus. Oncogene **24:**7673-85.
9. **Blanchette, P., C. Y. Cheng, Q. Yan, G. Ketner, D. A. Ornelles, T. Dobner, R. C. Conaway, J. W. Conaway, and P. E. Branton.** 2004. Both BC-box motifs of adenovirus protein E4orf6 are required to efficiently assemble an E3 ligase complex that degrades p53. Mol Cell Biol **24:**9619-29.
10. **Boutell, C., M. Canning, A. Orr, and R. D. Everett.** 2005. Reciprocal activities between herpes simplex virus type 1 regulatory protein ICP0, a ubiquitin E3 ligase, and ubiquitin-specific protease USP7. J Virol **79:**12342-54.
11. **Boutell, C., and R. D. Everett.** 2003. The herpes simplex virus type 1 (HSV-1) regulatory protein ICP0 interacts with and Ubiquitinates p53. J Biol Chem **278:**36596-602.
12. **Boutell, C., A. Orr, and R. D. Everett.** 2003. PML residue lysine 160 is required for the degradation of PML induced by herpes simplex virus type 1 regulatory protein ICP0. J Virol **77:**8686-94.
13. **Boutell, C., S. Sadis, and R. D. Everett.** 2002. Herpes simplex virus type 1 immediate-early protein ICP0 and is isolated RING finger domain act as ubiquitin E3 ligases in vitro. J Virol **76:**841-50.
14. **Burgert, H. G., Z. Ruzsics, S. Obermeier, A. Hilgendorf, M. Windheim, and A. Elsing.** 2002. Subversion of host defense mechanisms by adenoviruses. Curr Top Microbiol Immunol **269:**273-318.
15. **Camus, S., M. Higgins, D. P. Lane, and S. Lain.** 2003. Differences in the ubiquitination of p53 by Mdm2 and the HPV protein E6. FEBS Lett **536:**220-4.
16. **Canning, M., C. Boutell, J. Parkinson, and R. D. Everett.** 2004. A RING finger ubiquitin ligase is protected from autocatalyzed ubiquitination and degradation by binding to ubiquitin-specific protease USP7. J Biol Chem **279:**38160-8.
17. **Castro, A., C. Bernis, S. Vigneron, J. C. Labbe, and T. Lorca.** 2005. The anaphase-promoting complex: a key factor in the regulation of cell cycle. Oncogene **24:**314-25.
18. **Cathomen, T., and M. D. Weitzman.** 2000. A functional complex of adenovirus proteins E1B-55kDa and E4orf6 is necessary to modulate the expression level of p53 but not its transcriptional activity. J Virol **74:**11407-12.
19. **Chee, A. V., P. Lopez, P. P. Pandolfi, and B. Roizman.** 2003. Promyelocytic leukemia protein mediates interferon-based anti-herpes simplex virus 1 effects. J Virol **77:**7101-5.
20. **Chelbi-Alix, M. K., and H. de The.** 1999. Herpes virus induced proteasome-dependent degradation of the nuclear bodies-associated PML and Sp100 proteins. Oncogene **18:**935-41.
21. **Chen, J. J., C. E. Reid, V. Band, and E. J. Androphy.** 1995. Interaction of papillomavirus E6 oncoproteins with a putative calcium-binding protein. Science **269:**529-31.

22. **Chen, M., J. C. Cortay, I. R. Logan, V. Sapountzi, C. N. Robson, and D. Gerlier.** 2005. Inhibition of ubiquitination and stabilization of human ubiquitin E3 ligase PIRH2 by measles virus phosphoprotein. J Virol **79:**11824-36.
23. **Ciechanover, A., and R. Ben-Saadon.** 2004. N-terminal ubiquitination: more protein substrates join in. Trends Cell Biol **14:**103-6.
24. **Coscoy, L., D. J. Sanchez, and D. Ganem.** 2001. A novel class of herpesvirus-encoded membrane-bound E3 ubiquitin ligases regulates endocytosis of proteins involved in immune recognition. J Cell Biol **155:**1265-73.
25. **Craig, K. L., and M. Tyers.** 1999. The F-box: a new motif for ubiquitin dependent proteolysis in cell cycle regulation and signal transduction. Prog Biophys Mol Biol **72:**299-328.
26. **Crook, T., C. Fisher, P. J. Masterson, and K. H. Vousden.** 1994. Modulation of transcriptional regulatory properties of p53 by HPV E6. Oncogene **9:**1225-30.
27. **Didcock, L., D. F. Young, S. Goodbourn, and R. E. Randall.** 1999. The V protein of simian virus 5 inhibits interferon signalling by targeting STAT1 for proteasome-mediated degradation. J Virol **73:**9928-33.
28. **Dobner, T., N. Horikoshi, S. Rubenwolf, and T. Shenk.** 1996. Blockage by adenovirus E4orf6 of transcriptional activation by the p53 tumor suppressor. Science **272:**1470-3.
29. **Doherty, P. C., and S. J. Turner.** 2005. The virus-immunity ecosystem. Arch Virol Suppl**:**17-32.
30. **Dussart, S., M. Douaisi, M. Courcoul, G. Bessou, R. Vigne, and E. Decroly.** 2005. APOBEC3G ubiquitination by Nedd4-1 favors its packaging into HIV-1 particles. J Mol Biol **345:**547-58.
31. **Dyson, N., P. M. Howley, K. Munger, and E. Harlow.** 1989. The human papilloma virus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. Science **243:**934-7.
32. **Eidson, K. M., W. E. Hobbs, B. J. Manning, P. Carlson, and N. A. DeLuca.** 2002. Expression of herpes simplex virus ICP0 inhibits the induction of interferon-stimulated genes by viral infection. J Virol **76:**2180-91.
33. **Everett, R. D.** 2004. Herpes simplex virus type 1 regulatory protein ICP0 does not protect cyclins D1 and D3 from degradation during infection. J Virol **78:**9599-604.
34. **Everett, R. D., W. C. Earnshaw, J. Findlay, and P. Lomonte.** 1999. Specific destruction of kinetochore protein CENP-C and disruption of cell division by herpes simplex virus immediate-early protein Vmw110. Embo J **18:**1526-38.
35. **Everett, R. D., M. Meredith, A. Orr, A. Cross, M. Kathoria, and J. Parkinson.** 1997. A novel ubiquitin-specific protease is dynamically associated with the PML nuclear domain and binds to a herpesvirus regulatory protein. Embo J **16:**1519-30.
36. **Favre-Bonvin, A., C. Reynaud, C. Kretz-Remy, and P. Jalinot.** 2005. Human papillomavirus type 18 E6 protein binds the cellular PDZ protein TIP-2/GIPC, which is involved in transforming growth factor beta signaling and triggers its degradation by the proteasome. J Virol **79:**4229-37.
37. **Fehrmann, F., and L. A. Laimins.** 2003. Human papillomaviruses: targeting differentiating epithelial cells for malignant transformation. Oncogene **22:**5201-7.
38. **Finlay, B. B., and G. McFadden.** 2006. Anti-immunology: evasion of the host immune system by bacterial and viral pathogens. Cell **124:**767-82.
39. **Finley, D., A. Ciechanover, and A. Varshavsky.** 2004. Ubiquitin as a central cellular regulator. Cell **116:**S29-32, 2 p following S32.
40. **Fujita, M., H. Akari, A. Sakurai, A. Yoshida, T. Chiba, K. Tanaka, K. Strebel, and A. Adachi.** 2004. Expression of HIV-1 accessory protein Vif is controlled uniquely to be low and optimal by proteasome degradation. Microbes Infect **6:**791-8.
41. **Gage, J. R., C. Meyers, and F. O. Wettstein.** 1990. The E7 proteins of the nononcogenic human papillomavirus type 6b (HPV-6b) and of the oncogenic HPV-16 differ in retinoblastoma protein binding and other properties. J Virol **64:**723-30.
42. **Gross-Mesilaty, S., E. Reinstein, B. Bercovich, K. E. Tobias, A. L. Schwartz, C. Kahana, and A. Ciechanover.** 1998. Basal and human papillomavirus E6 oncoprotein-induced degradation of Myc proteins by the ubiquitin pathway. Proc Natl Acad Sci U S A **95:**8058-63.
43. **Gu, H., and B. Roizman.** 2003. The degradation of promyelocytic leukemia and Sp100 proteins by herpes simplex virus 1 is mediated by the ubiquitin-conjugating enzyme UbcH5a. Proc Natl Acad Sci U S A **100:**8963-8.

44. **Gu, J., R. M. Rubin, and Z. M. Yuan.** 2001. A sequence element of p53 that determines its susceptibility to viral oncoprotein-targeted degradation. Oncogene **20:**3519-27.
45. **Gupta, S., P. P. Takhar, R. Degenkolbe, C. H. Koh, H. Zimmermann, C. M. Yang, K. Guan Sim, S. I. Hsu, and H. U. Bernard.** 2003. The human papillomavirus type 11 and 16 E6 proteins modulate the cell-cycle regulator and transcription cofactor TRIP-Br1. Virology **317:**155-64.
46. **Hagglund, R., and B. Roizman.** 2004. Role of ICP0 in the strategy of conquest of the host cell by herpes simplex virus 1. J Virol **78:**2169-78.
47. **Hagglund, R., C. Van Sant, P. Lopez, and B. Roizman.** 2002. Herpes simplex virus 1-infected cell protein 0 contains two E3 ubiquitin ligase sites specific for different E2 ubiquitin-conjugating enzymes. Proc Natl Acad Sci U S A **99:**631-6.
48. **Harada, J. N., A. Shevchenko, A. Shevchenko, D. C. Pallas, and A. J. Berk.** 2002. Analysis of the adenovirus E1B-55K-anchored proteome reveals its link to ubiquitination machinery. J Virol **76:**9194-206.
49. **Harle, P., B. Sainz, Jr., D. J. Carr, and W. P. Halford.** 2002. The immediate-early protein, ICP0, is essential for the resistance of herpes simplex virus to interferon-alpha/beta. Virology **293:**295-304.
50. **Hatakeyama, S., and K. I. Nakayama.** 2003. U-box proteins as a new family of ubiquitin ligases. Biochem Biophys Res Commun **302:**635-45.
51. **He, B., R. G. Paterson, N. Stock, J. E. Durbin, R. K. Durbin, S. Goodbourn, R. E. Randall, and R. A. Lamb.** 2002. Recovery of paramyxovirus simian virus 5 with a V protein lacking the conserved cysteine-rich domain: the multifunctional V protein blocks both interferon-beta induction and interferon signaling. Virology **303:**15-32.
52. **Hemelaar, J., A. Borodovsky, B. M. Kessler, D. Reverter, J. Cook, N. Kolli, T. Gan-Erdene, K. D. Wilkinson, G. Gill, C. D. Lima, H. L. Ploegh, and H. Ovaa.** 2004. Specific and covalent targeting of conjugating and deconjugating enzymes of ubiquitin-like proteins. Mol Cell Biol **24:**84-95.
53. **Hershko, A., and A. Ciechanover.** 1998. The ubiquitin system. Annu Rev Biochem **67:**425-79.
54. **Hobbs, W. E., D. E. Brough, I. Kovesdi, and N. A. DeLuca.** 2001. Efficient activation of viral genomes by levels of herpes simplex virus ICP0 insufficient to affect cellular gene expression or cell survival. J Virol **75:**3391-403.
55. **Huibregtse, J. M., M. Scheffner, and P. M. Howley.** 1993. Cloning and expression of the cDNA for E6-AP, a protein that mediates the interaction of the human papillomavirus E6 oncoprotein with p53. Mol Cell Biol **13:**775-84.
56. **Huibregtse, J. M., M. Scheffner, and P. M. Howley.** 1993. Localization of the E6-AP regions that direct human papillomavirus E6 binding, association with p53, and ubiquitination of associated proteins. Mol Cell Biol **13:**4918-27.
57. **Iannello, A., O. Debbeche, E. Martin, L. H. Attalah, S. Samarani, and A. Ahmad.** 2006. Viral strategies for evading antiviral cellular immune responses of the host. J Leukoc Biol **79:**16-35.
58. **Ingham, R. J., G. Gish, and T. Pawson.** 2004. The Nedd4 family of E3 ubiquitin ligases: functional diversity within a common modular architecture. Oncogene **23:**1972-84.
59. **Kao, W. H., S. L. Beaudenon, A. L. Talis, J. M. Huibregtse, and P. M. Howley.** 2000. Human papillomavirus type 16 E6 induces self-ubiquitination of the E6AP ubiquitin-protein ligase. J Virol **74:**6408-17.
60. **Karpova, A. Y., M. Trost, J. M. Murray, L. C. Cantley, and P. M. Howley.** 2002. Interferon regulatory factor-3 is an in vivo target of DNA-PK. Proc Natl Acad Sci U S A **99:**2818-23.
61. **Kobayashi, M., A. Takaori-Kondo, Y. Miyauchi, K. Iwai, and T. Uchiyama.** 2005. Ubiquitination of APOBEC3G by an HIV-1 Vif-Cullin5-Elongin B-Elongin C complex is essential for Vif function. J Biol Chem **280:**18573-8.
62. **Koromilas, A. E., S. Li, and G. Matlashewski.** 2001. Control of interferon signaling in human papillomavirus infection. Cytokine Growth Factor Rev **12:**157-70.
63. **Leib, D. A., T. E. Harrison, K. M. Laslo, M. A. Machalek, N. J. Moorman, and H. W. Virgin.** 1999. Interferons regulate the phenotype of wild-type and mutant herpes simplex viruses in vivo. J Exp Med **189:**663-72.
64. **Li, M., C. L. Brooks, N. Kon, and W. Gu.** 2004. A dynamic role of HAUSP in the p53-Mdm2 pathway. Mol Cell **13:**879-86.

65. **Li, S., S. Labrecque, M. C. Gauzzi, A. R. Cuddihy, A. H. Wong, S. Pellegrini, G. J. Matlashewski, and A. E. Koromilas.** 1999. The human papilloma virus (HPV)-18 E6 oncoprotein physically associates with Tyk2 and impairs Jak-STAT activation by interferon-alpha. Oncogene **18:**5727-37.

66. **Li, T., X. Chen, K. C. Garbutt, P. Zhou, and N. Zheng.** 2006. Structure of DDB1 in complex with a paramyxovirus V protein: viral hijack of a propeller cluster in ubiquitin ligase. Cell **124:**105-17.

67. **Li, T. T., L. N. Zhao, Z. G. Liu, Y. Han, and D. M. Fan.** 2005. Regulation of apoptosis by the papillomavirus E6 oncogene. World J Gastroenterol **11:**931-7.

68. **Lin, G. Y., R. G. Paterson, C. D. Richardson, and R. A. Lamb.** 1998. The V protein of the paramyxovirus SV5 interacts with damage-specific DNA binding protein. Virology **249:**189-200.

69. **Lin, R., R. S. Noyce, S. E. Collins, R. D. Everett, and K. L. Mossman.** 2004. The herpes simplex virus ICP0 RING finger domain inhibits IRF3- and IRF7-mediated activation of interferon-stimulated genes. J Virol **78:**1675-84.

70. **Lin, Y., F. Horvath, J. A. Aligo, R. Wilson, and B. He.** 2005. The role of simian virus 5 V protein on viral RNA synthesis. Virology **338:**270-80.

71. **Liu, B., P. T. Sarkis, K. Luo, Y. Yu, and X. F. Yu.** 2005. Regulation of Apobec3F and human immunodeficiency virus type 1 Vif by Vif-Cul5-ElonB/C E3 ubiquitin ligase. J Virol **79:**9579-87.

72. **Liu, X., H. Yuan, B. Fu, G. L. Disbrow, T. Apolinario, V. Tomaic, M. L. Kelley, C. C. Baker, J. Huibregtse, and R. Schlegel.** 2005. The E6AP ubiquitin ligase is required for transactivation of the hTERT promoter by the human papillomavirus E6 oncoprotein. J Biol Chem **280:**10807-16.

73. **Liu, Y., A. L. Colosimo, X. J. Yang, and D. Liao.** 2000. Adenovirus E1B 55-kilodalton oncoprotein inhibits p53 acetylation by PCAF. Mol Cell Biol **20:**5540-53.

74. **Liu, Y., A. Shevchenko, A. Shevchenko, and A. J. Berk.** 2005. Adenovirus exploits the cellular aggresome response to accelerate inactivation of the MRN complex. J Virol **79:**14004-16.

75. **Liu, Y. C.** 2004. Ubiquitin ligases and the immune response. Annu Rev Immunol **22:**81-127.

76. **Lomonte, P., and R. D. Everett.** 1999. Herpes simplex virus type 1 immediate-early protein Vmw110 inhibits progression of cells through mitosis and from G(1) into S phase of the cell cycle. J Virol **73:**9456-67.

77. **Lomonte, P., K. F. Sullivan, and R. D. Everett.** 2001. Degradation of nucleosome-associated centromeric histone H3-like protein CENP-A induced by herpes simplex virus type 1 protein ICP0. J Biol Chem **276:**5829-35.

78. **Luo, K., Z. Xiao, E. Ehrlich, Y. Yu, B. Liu, S. Zheng, and X. F. Yu.** 2005. Primate lentiviral virion infectivity factors are substrate receptors that assemble with cullin 5-E3 ligase through a HCCH motif to suppress APOBEC3G. Proc Natl Acad Sci U S A **102:**11444-9.

79. **Martin, M. E., and A. J. Berk.** 1998. Adenovirus E1B 55K represses p53 activation in vitro. J Virol **72:**3146-54.

80. **Mehle, A., J. Goncalves, M. Santa-Marta, M. McPike, and D. Gabuzda.** 2004. Phosphorylation of a novel SOCS-box regulates assembly of the HIV-1 Vif-Cul5 complex that promotes APOBEC3G degradation. Genes Dev **18:**2861-6.

81. **Melroe, G. T., N. A. DeLuca, and D. M. Knipe.** 2004. Herpes simplex virus 1 has multiple mechanisms for blocking virus-induced interferon production. J Virol **78:**8411-20.

82. **Mossman, K. L., P. F. Macgregor, J. J. Rozmus, A. B. Goryachev, A. M. Edwards, and J. R. Smiley.** 2001. Herpes simplex virus triggers and then disarms a host antiviral response. J Virol **75:**750-8.

83. **Mossman, K. L., H. A. Saffran, and J. R. Smiley.** 2000. Herpes simplex virus ICP0 mutants are hypersensitive to interferon. J Virol **74:**2052-6.

84. **Mossman, K. L., and J. R. Smiley.** 2002. Herpes simplex virus ICP0 and ICP34.5 counteract distinct interferon-induced barriers to virus replication. J Virol **76:**1995-8.

85. **Nees, M., J. M. Geoghegan, T. Hyman, S. Frank, L. Miller, and C. D. Woodworth.** 2001. Papillomavirus type 16 oncogenes downregulate expression of interferon-responsive genes and upregulate proliferation-associated and NF-kappaB-responsive genes in cervical keratinocytes. J Virol **75:**4283-96.

86. **Newman, E. N., R. K. Holmes, H. M. Craig, K. C. Klein, J. R. Lingappa, M. H. Malim, and A. M. Sheehy.** 2005. Antiviral function of APOBEC3G can be dissociated from cytidine deaminase activity. Curr Biol **15:**166-70.

87. **Nicholl, M. J., L. H. Robinson, and C. M. Preston.** 2000. Activation of cellular interferon-responsive genes after infection of human cells with herpes simplex virus type 1. J Gen Virol **81:**2215-8.

88. **Nyman, T. A., S. Matikainen, T. Sareneva, I. Julkunen, and N. Kalkkinen.** 2000. Proteome analysis reveals ubiquitin-conjugating enzymes to be a new family of interferon-alpha-regulated genes. Eur J Biochem **267:**4011-9.

89. **Oh, K. J., A. Kalinina, J. Wang, K. Nakayama, K. I. Nakayama, and S. Bagchi.** 2004. The papillomavirus E7 oncoprotein is ubiquitinated by UbcH7 and Cullin 1- and Skp2-containing E3 ligase. J Virol **78:**5338-46.

90. **Ohh, M., W. Y. Kim, J. J. Moslehi, Y. Chen, V. Chau, M. A. Read, and W. G. Kaelin, Jr.** 2002. An intact NEDD8 pathway is required for Cullin-dependent ubiquitylation in mammalian cells. EMBO Rep **3:**177-82.

91. **Okumura, A., G. Lu, I. Pitha-Rowe, and P. M. Pitha.** 2006. Innate antiviral response targets HIV-1 release by the induction of ubiquitin-like protein ISG15. Proc Natl Acad Sci U S A **103:**1440-5.

92. **Parkinson, J., and R. D. Everett.** 2000. Alphaherpesvirus proteins related to herpes simplex virus type 1 ICP0 affect cellular structures and proteins. J Virol **74:**10006-17.

93. **Parkinson, J., S. P. Lees-Miller, and R. D. Everett.** 1999. Herpes simplex virus type 1 immediate-early protein vmw110 induces the proteasome-dependent degradation of the catalytic subunit of DNA-dependent protein kinase. J Virol **73:**650-7.

94. **Patel, D., S. M. Huang, L. A. Baglia, and D. J. McCance.** 1999. The E6 protein of human papillomavirus type 16 binds to and inhibits co-activation by CBP and p300. Embo J **18:**5061-72.

95. **Perry, A. K., G. Chen, D. Zheng, H. Tang, and G. Cheng.** 2005. The host type I interferon response to viral and bacterial infections. Cell Res **15:**407-22.

96. **Pim, D., A. Storey, M. Thomas, P. Massimi, and L. Banks.** 1994. Mutational analysis of HPV-18 E6 identifies domains required for p53 degradation in vitro, abolition of p53 transactivation in vivo and immortalisation of primary BMK cells. Oncogene **9:**1869-76.

97. **Porta, C., R. Hadj-Slimane, M. Nejmeddine, M. Pampin, M. G. Tovey, L. Espert, S. Alvarez, and M. K. Chelbi-Alix.** 2005. Interferons alpha and gamma induce p53-dependent and p53-independent apoptosis, respectively. Oncogene **24:**605-15.

98. **Precious, B., K. Childs, V. Fitzpatrick-Swallow, S. Goodbourn, and R. E. Randall.** 2005. Simian virus 5 V protein acts as an adaptor, linking DDB1 to STAT2, to facilitate the ubiquitination of STAT1. J Virol **79:**13434-41.

99. **Preston, C. M., A. N. Harman, and M. J. Nicholl.** 2001. Activation of interferon response factor-3 in human cells infected with herpes simplex virus type 1 or human cytomegalovirus. J Virol **75:**8909-16.

100. **Querido, E., P. Blanchette, Q. Yan, T. Kamura, M. Morrison, D. Boivin, W. G. Kaelin, R. C. Conaway, J. W. Conaway, and P. E. Branton.** 2001. Degradation of p53 by adenovirus E4orf6 and E1B55K proteins occurs via a novel mechanism involving a Cullin-containing complex. Genes Dev **15:**3104-17.

101. **Querido, E., R. C. Marcellus, A. Lai, R. Charbonneau, J. G. Teodoro, G. Ketner, and P. E. Branton.** 1997. Regulation of p53 levels by the E1B 55-kilodalton protein and E4orf6 in adenovirus-infected cells. J Virol **71:**3788-98.

102. **Querido, E., M. R. Morrison, H. Chu-Pham-Dang, S. W. Thirlwell, D. Boivin, and P. E. Branton.** 2001. Identification of three functions of the adenovirus e4orf6 protein that mediate p53 degradation by the E4orf6-E1B55K complex. J Virol **75:**699-709.

103. **Ronco, L. V., A. Y. Karpova, M. Vidal, and P. M. Howley.** 1998. Human papillomavirus 16 E6 oncoprotein binds to interferon regulatory factor-3 and inhibits its transcriptional activity. Genes Dev **12:**2061-72.

104. **Rose, K. M., M. Marin, S. L. Kozak, and D. Kabat.** 2004. The viral infectivity factor (Vif) of HIV-1 unveiled. Trends Mol Med **10:**291-7.

105. **Roth, J., and M. Dobbelstein.** 2003. Interaction of p53 with the adenovirus E1B-55 kDa protein. Methods Mol Biol **234:**135-49.

106. **Salomoni, P., and P. P. Pandolfi.** 2002. The role of PML in tumor suppression. Cell **108:**165-70.

107. **Sang, B. C., and M. S. Barbosa.** 1992. Single amino acid substitutions in "low-risk" human papillomavirus (HPV) type 6 E7 protein enhance features characteristic of the "high-risk" HPV E7 oncoproteins. Proc Natl Acad Sci U S A **89:**8063-7.

108. **Santa-Marta, M., F. A. da Silva, A. M. Fonseca, and J. Goncalves.** 2005. HIV-1 Vif can directly inhibit apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3G-mediated cytidine deamination by using a single amino acid interaction and without protein degradation. J Biol Chem **280:**8765-75.

109. **Scheel, H., and K. Hofmann.** 2003. No evidence for PHD fingers as ubiquitin ligases. Trends Cell Biol **13:**285-7; author reply 287-8.

110. **Scheffner, M., and N. J. Whitaker.** 2003. Human papillomavirus-induced carcinogenesis and the ubiquitin-proteasome system. Semin Cancer Biol **13:**59-67.

111. **Sobol, P. T., and K. L. Mossman.** 2006. ICP0 Prevents RNase L-Independent rRNA Cleavage in Herpes Simplex Virus Type 1-Infected Cells. J Virol **80:**218-25.

112. **Steegenga, W. T., N. Riteco, A. G. Jochemsen, F. J. Fallaux, and J. L. Bos.** 1998. The large E1B protein together with the E4orf6 protein target p53 for active degradation in adenovirus infected cells. Oncogene **16:**349-57.

113. **Stephanou, A., and D. S. Latchman.** 2005. Opposing actions of STAT-1 and STAT-3. Growth Factors **23:**177-82.

114. **Stracker, T. H., C. T. Carson, and M. D. Weitzman.** 2002. Adenovirus oncoproteins inactivate the Mre11-Rad50-NBS1 DNA repair complex. Nature **418:**348-52.

115. **Sun, M., T. A. Rothermel, L. Shuman, J. A. Aligo, S. Xu, Y. Lin, R. A. Lamb, and B. He.** 2004. Conserved cysteine-rich domain of paramyxovirus simian virus 5 V protein plays an important role in blocking apoptosis. J Virol **78:**5068-78.

116. **Sun, R., S. F. Lin, L. Gradoville, Y. Yuan, F. Zhu, and G. Miller.** 1998. A viral gene that activates lytic cycle expression of Kaposi's sarcoma-associated herpesvirus. Proc Natl Acad Sci U S A **95:**10866-71.

117. **Talis, A. L., J. M. Huibregtse, and P. M. Howley.** 1998. The role of E6AP in the regulation of p53 protein levels in human papillomavirus (HPV)-positive and HPV-negative cells. J Biol Chem **273:**6439-45.

118. **Tanaka, Y., H. Marusawa, H. Seno, Y. Matsumoto, Y. Ueda, Y. Kodama, Y. Endo, J. Yamauchi, T. Matsumoto, A. Takaori-Kondo, I. Ikai, and T. Chiba.** 2006. Anti-viral protein APOBEC3G is induced by interferon-alpha stimulation in human hepatocytes. Biochem Biophys Res Commun **341:**314-9.

119. **Ulane, C. M., and C. M. Horvath.** 2002. Paramyxoviruses SV5 and HPIV2 assemble STAT protein ubiquitin ligase complexes from cellular components. Virology **304:**160-6.

120. **Ulane, C. M., A. Kentsis, C. D. Cruz, J. P. Parisien, K. L. Schneider, and C. M. Horvath.** 2005. Composition and assembly of STAT-targeting ubiquitin ligase complexes: paramyxovirus V protein carboxyl terminus is an oligomerization domain. J Virol **79:**10180-9.

121. **Van Sant, C., R. Hagglund, P. Lopez, and B. Roizman.** 2001. The infected cell protein 0 of herpes simplex virus 1 dynamically interacts with proteasomes, binds and activates the cdc34 E2 ubiquitin-conjugating enzyme, and possesses in vitro E3 ubiquitin ligase activity. Proc Natl Acad Sci U S A **98:**8815-20.

122. **Veldman, T., X. Liu, H. Yuan, and R. Schlegel.** 2003. Human papillomavirus E6 and Myc proteins associate in vivo and bind to and cooperatively activate the telomerase reverse transcriptase promoter. Proc Natl Acad Sci U S A **100:**8211-6.

123. **Werness, B. A., A. J. Levine, and P. M. Howley.** 1990. Association of human papillomavirus types 16 and 18 E6 proteins with p53. Science **248:**76-9.

124. **Wilkinson, K. D.** 2000. Ubiquitination and deubiquitination: targeting of proteins for degradation by the proteasome. Semin Cell Dev Biol **11:**141-8.

125. **Young, D. F., N. Chatziandreou, B. He, S. Goodbourn, R. A. Lamb, and R. E. Randall.** 2001. Single amino acid substitution in the V protein of simian virus 5 differentiates its ability to block interferon signaling in human and murine cells. J Virol **75:**3363-70.

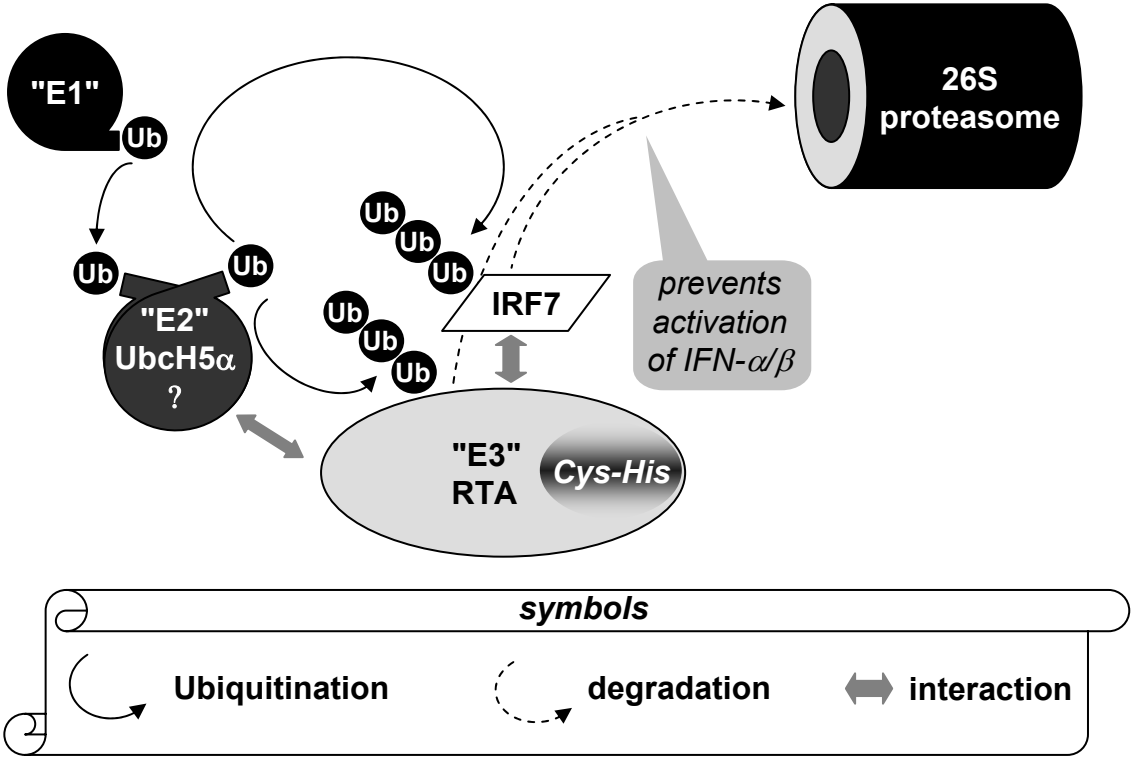
126. **Yu, X., Y. Yu, B. Liu, K. Luo, W. Kong, P. Mao, and X. F. Yu.** 2003. Induction of APOBEC3G ubiquitination and degradation by an HIV-1 Vif-Cul5-SCF complex. Science **302:**1056-60.

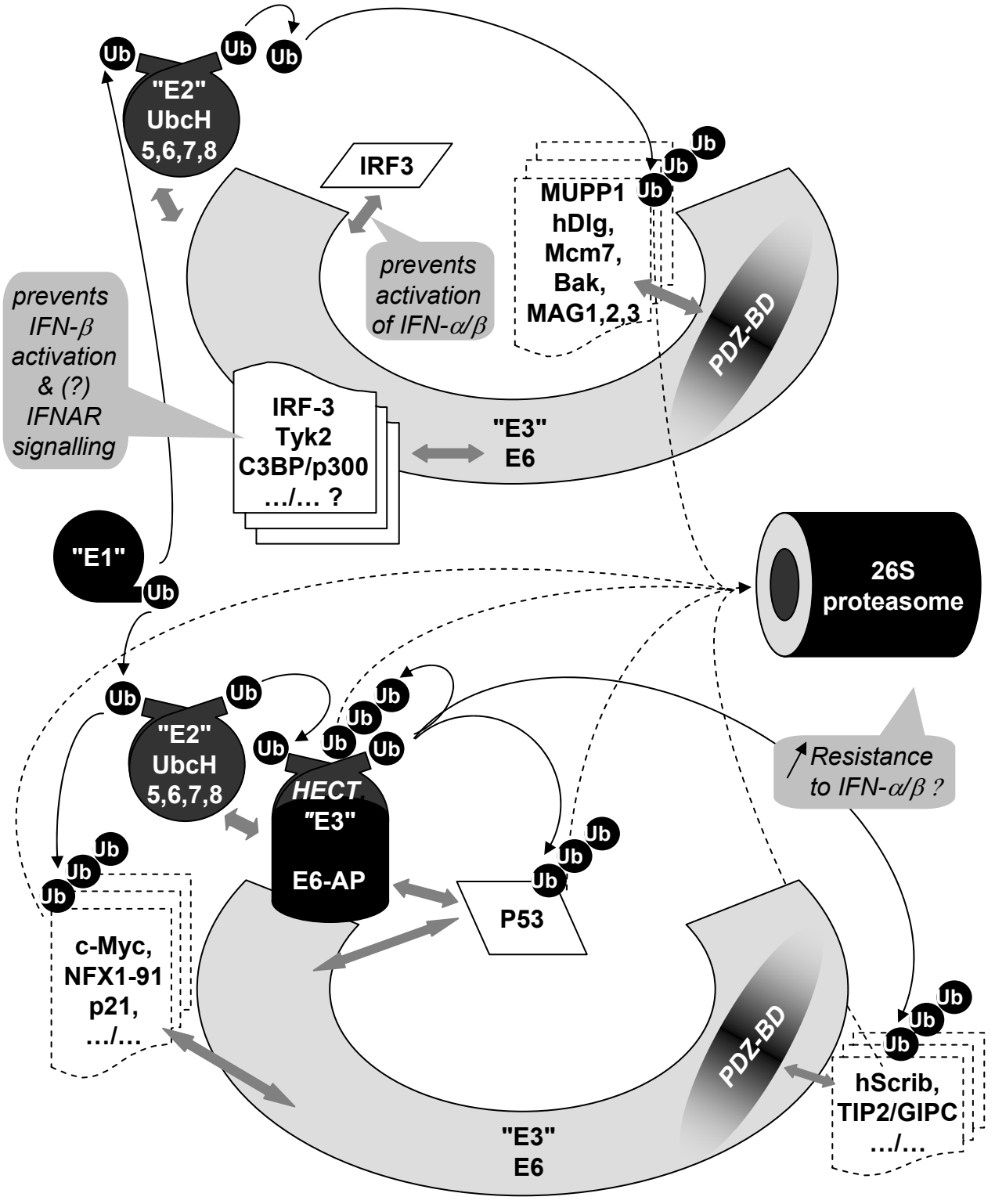
127. **Yu, Y., S. E. Wang, and G. S. Hayward.** 2005. The KSHV immediate-early transcription factor RTA encodes ubiquitin E3 ligase activity that targets IRF7 for proteosome-mediated degradation. Immunity **22:**59-70.

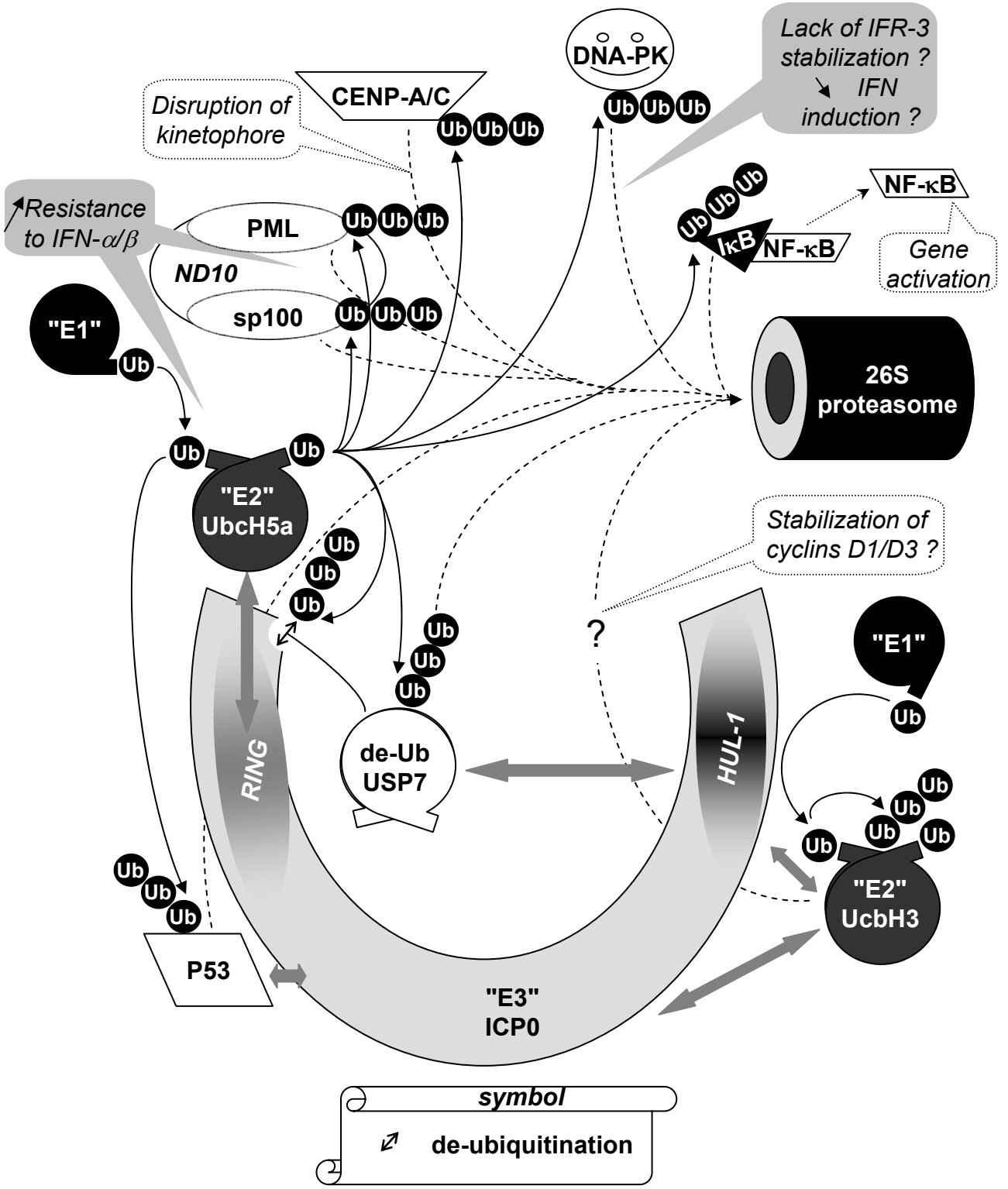
128. **Yu, Y., Z. Xiao, E. S. Ehrlich, X. Yu, and X. F. Yu.** 2004. Selective assembly of HIV-1 Vif-Cul5-ElonginB-ElonginC E3 ubiquitin ligase complex through a novel SOCS box and upstream cysteines. Genes Dev **18:**2867-72.

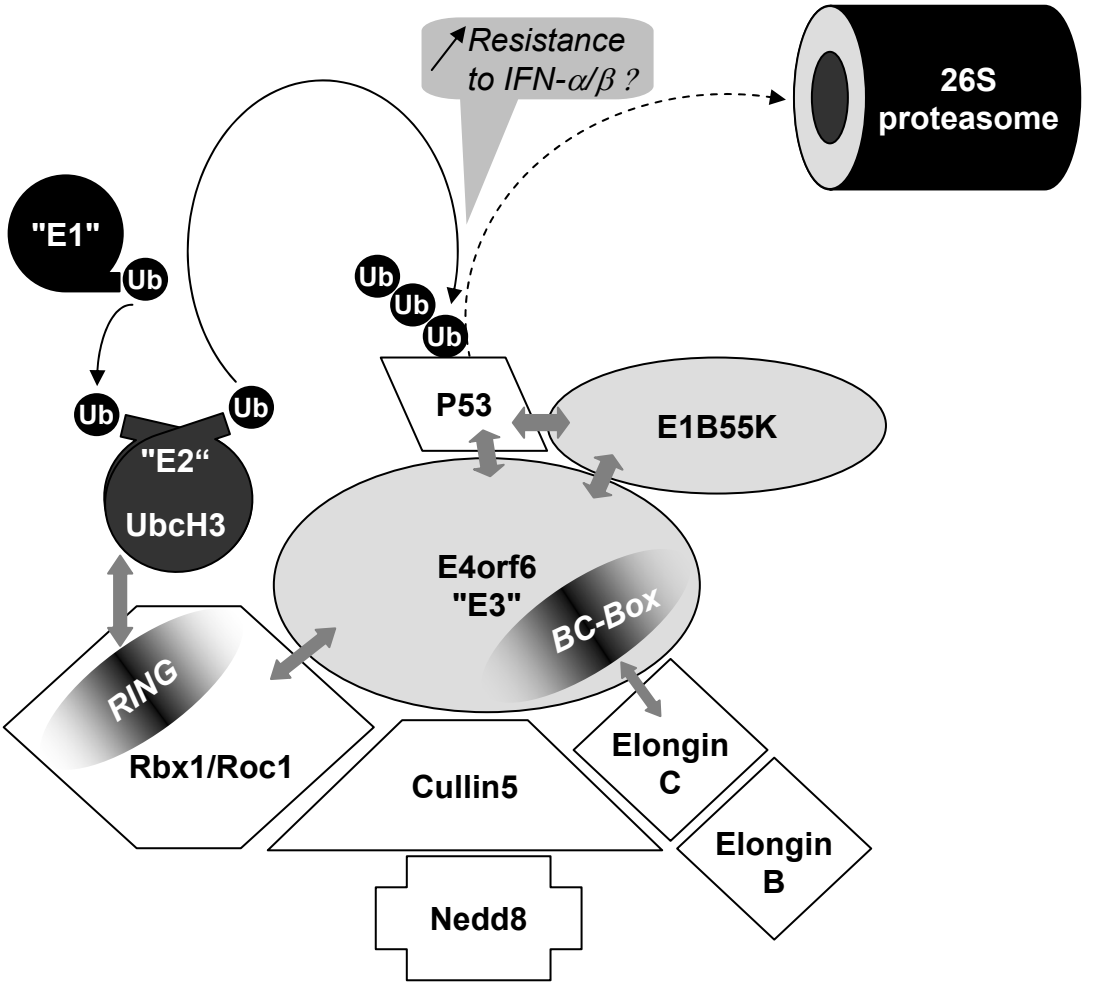
129. **Yuan, W., and R. M. Krug.** 2001. Influenza B virus NS1 protein inhibits conjugation of the interferon (IFN)-induced ubiquitin-like ISG15 protein. Embo J **20:**362-71.

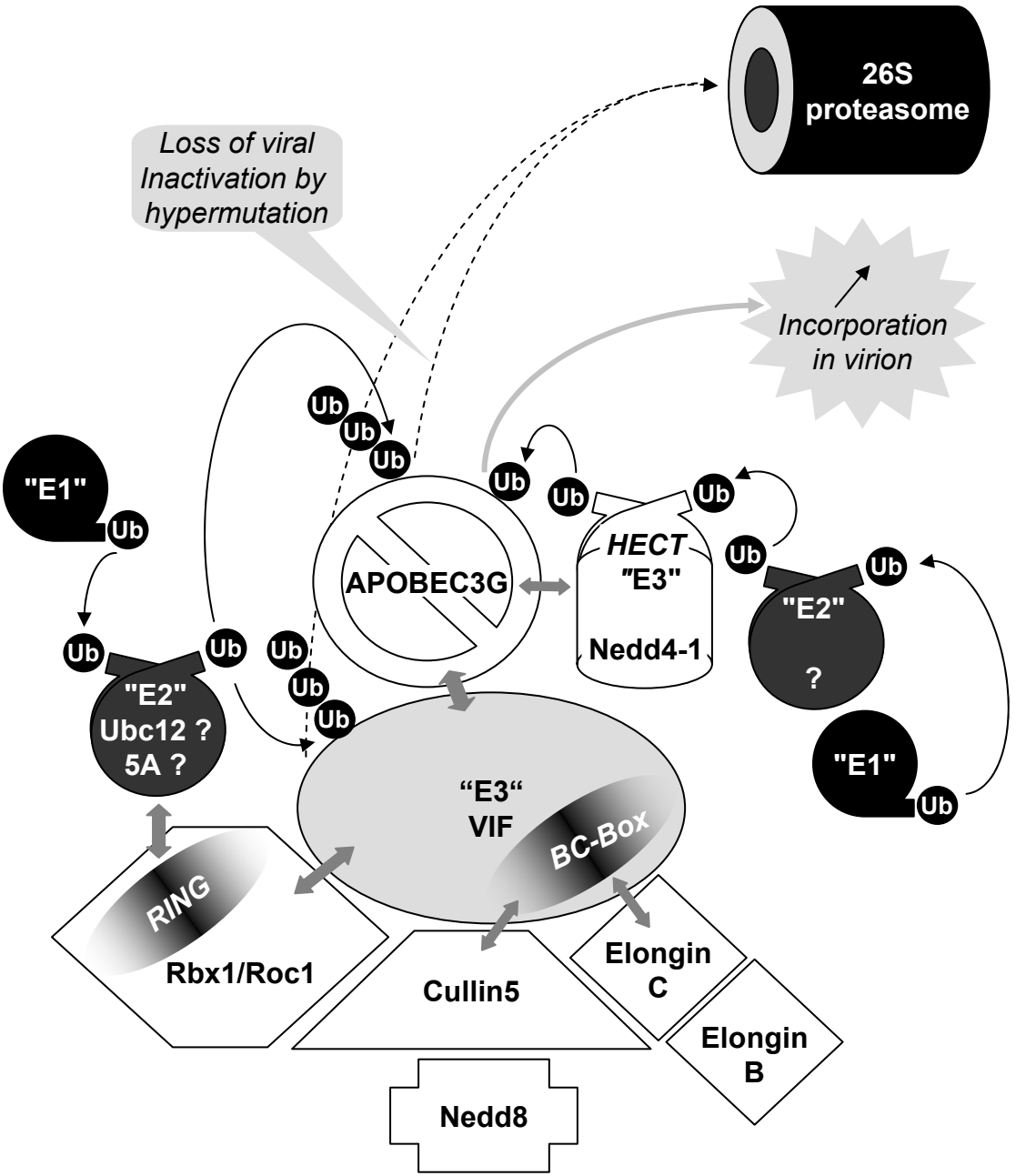
130. **Zimmermann, H., C. H. Koh, R. Degenkolbe, M. J. O'Connor, A. Muller, G. Steger, J. J. Chen, Y. Lui, E. Androphy, and H. U. Bernard.** 2000. Interaction with CBP/p300 enables the bovine papillomavirus type 1 E6 oncoprotein to downregulate CBP/p300-mediated transactivation by p53. J Gen Virol **81:**2617-23.

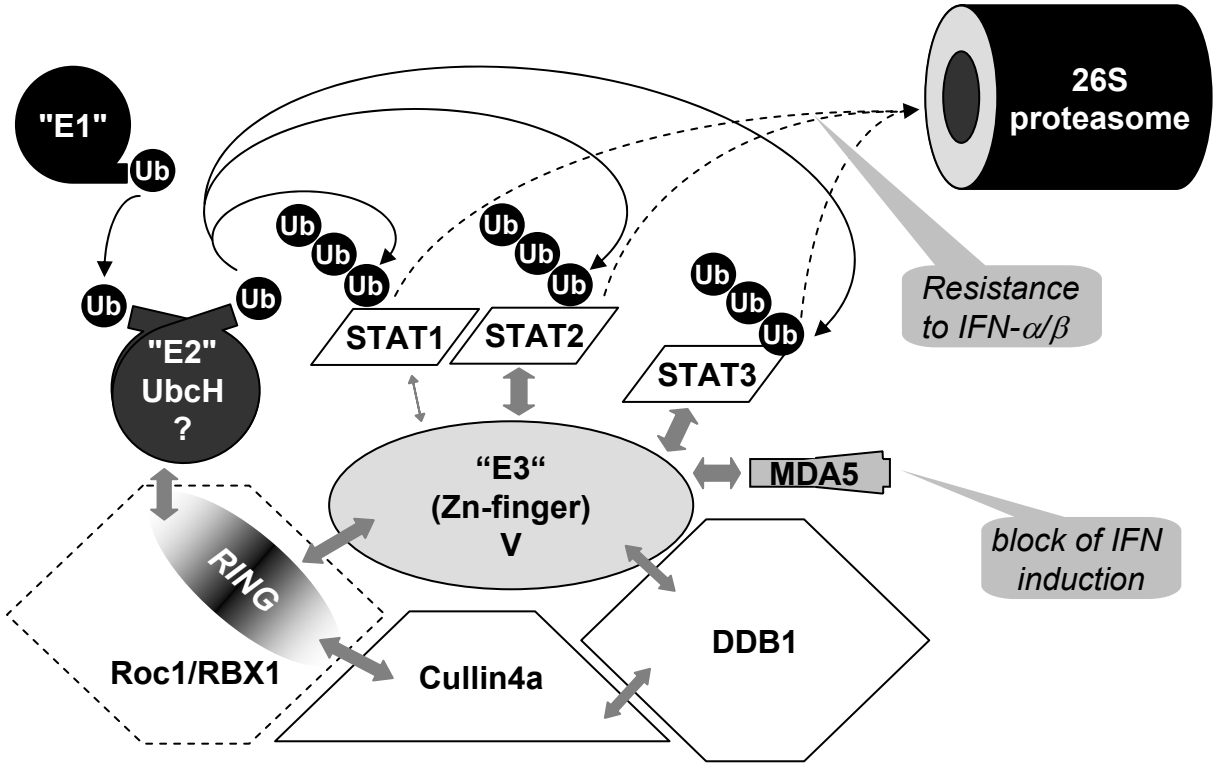

"E1"
Ub
"E2"
UbcH5α
?
IRF7
"E3"
RTA
Cys-His
26S
proteasome
prevents
activation
of IFN-α/β
symbols
Ubiquitination
degradation
interaction


"E2"
UbcH
5,6,7,8
Ub
IRF3
prevents
activation
of IFN-α/β
MUPP1
hDlg,
Mcm7,
Bak,
MAG1,2,3
PDZ-BD
prevents
IFN-β
activation
& (?)
IFNAR
signalling
IRF-3
Tyk2
C3BP/p300
…/… ?
"E3"
E6
"E1"
26S
proteasome
HECT
"E3"
E6-AP
P53
c-Myc,
NFX1-91
p21,
…/…
Resistance
to IFN-α/β ?
hScrib,
TIP2/GIPC
…/…


DNA-PK
Lack of IFR-3 stabilization ?
IFN induction ?
CENP-A/C
Disruption of kinetophore
Resistance to IFN-α/β
PML
ND10
sp100
NF-κB
IκB
NF-κB
Gene activation
"E1"
Ub
26S proteasome
"E2" UbcH5a
Stabilization of cyclins D1/D3 ?
?
RING
de-Ub USP7
HUL-1
"E1"
"E2" UcbH3
P53
"E3" ICP0
symbol
de-ubiquitination


Resistance
to IFN-α/β ?
26S
proteasome
"E1"
Ub
Ub
Ub
Ub
Ub
Ub
"E2"
UbcH3
P53
E1B55K
E4orf6
"E3"
BC-Box
RING
Rbx1/Roc1
Cullin5
Elongin
C
Elongin
B
Nedd8


26S proteasome
Loss of viral Inactivation by hypermutation
Incorporation in virion
"E1"
Ub
"E2" Ubc12 ? 5A ?
APOBEC3G
HECT "E3" Nedd4-1
"E2" ?
"E1"
"E3" VIF
BC-Box
RING
Rbx1/Roc1
Cullin5
Elongin C
Elongin B
Nedd8


"E1"
Ub
26S
proteasome
Ub
Ub
Ub
Ub
Ub
Ub
Ub
Ub
Ub
Ub
Ub
"E2"
UbcH
?
STAT1
STAT2
STAT3
Resistance
to IFN-α/β
"E3"
(Zn-finger)
V
MDA5
block of IFN
induction
RING
Roc1/RBX1
Cullin4a
DDB1